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Influence of Ca^{2+} Ions and Cation Chelators on Activity Measurement of Creatine Kinase Isoenzymes

By *K. Jung, Regina Neumann, W. Liese* and *E. Egger*

Department of Clinical Biochemistry, University Hospital Charité, Humboldt University Berlin, Berlin and Department of Clinical Chemistry, Medical Academy Magdeburg, Magdeburg

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In commemoration of Professor Dr. Gábor Szász[1])

Summary: EDTA and EGTA, added to the reaction mixture for the activity determination of creatine kinase, stimulate the activity of creatine kinase to various extents by suppressing the inhibitory effect of Ca^{2+} ions. The activation effect is highest for isoenzyme BB, less for isoenzyme MB, and lowest for isoenzyme MM.

Zum Einfluß von Ca^{2+}-Ionen und Komplexbildnern auf die Aktivitätsbestimmung von Kreatinkinase-Isoenzymen

Zusammenfassung: EDTA und EGTA im Reaktionsansatz für die Bestimmung der Kreatinkinase-Aktivität stimulieren infolge Beseitigung des durch Ca^{2+}-Ionen bedingten Hemmeffekts die drei Isoenzyme der Kreatinkinase in einem unterschiedlichen Ausmaß. Das Isoenzym BB wird stärker als die Isoenzyme MM und MB aktiviert.

Introduction

The influence of cation chelators on activity measurements of creatine kinase (ATP: Creatin-N-Phosphotransferase, EC 2.7.3.2) was recently demonstrated (1, 2, 3, 4). It was found that the addition of chelators, such as ethylenediaminetetraacetic acid (EDTA) or ethyleneglycol-bis(2-aminoethylether)-N,N′-tetraacetic acid (EGTA) led to an activating effect upon creatine kinase activity in human serum, caused by the binding of inhibitory Ca^{2+} ions. When attempting to measure the creatine kinase isoenzymes in different diseases, it is necessary to know whether this effect of cation chelators varies from one creatine kinase isoenzyme to another. The aim of this study is to show the different influence of Ca^{2+} ions and cation chelators on the activity of the various isoenzymes of creatine kinase.

Materials and Methods

The activity measurements were performed under reaction conditions given by *Szasz* et al. (5,6) at 340 nm, 37°C with CentrifiChem 400 (t_0 = 180 s, Δt = 60 s; 5 points). The final concentrations in the mixture were 100 mmol/l imidazole buffer (pH 6.7), 30 mmol/l creatine phosphate, 2 mmol/l ADP, 5 mmol/l AMP, 20 mmol/l N-acetyl cysteine, 10 µmol/l diadenosine pentaphosphate, 20 mmol/l *D*-glucose, 2 mmol/l $NADP^+$, 10 mmol/l Mg^{2+}, 2500 U/l hexokinase (EC 2.7.1.1), 1500 U/l glucose-6-phosphate dehydrogenase (EC 1.1.1.43). The concentrations of EDTA, EGTA and Ca^{2+} are given in the figures. Ratio of sample volume to final volume was 1:26. The precision within series (CV) was 3.4% (mean test activity 99.5 U/l; S.D. 3.4 U/l; n=10). Pure isoenzymes of creatine kinase prepared from human heart, brain and muscle (7,8) were used for the investigation. All reagents and buffers were prepared with doubly distilled, deionized water. Imidazole and EGTA were obtained from Ferak, Berlin; EDTA from Berlin-Chemie, Berlin; $MgCl_2$ and $CaCl_2$ from Laborchemie Apolda; DEAE-Sephadex from Pharmacia, Uppsala; glucose from Polfa, Cracow. All other biochemicals were products of Boehringer Mannheim GmbH.

Results

We measured the effect of the Ca^{2+} concentration on the activities of the various isoenzymes of creatine kinase (fig. 1). Each isoenzyme of creatine kinase is inhibited to a different extent by Ca^{2+} ions. The isoenzyme BB is the most strongly inhibited, while the activities of isoenzymes MB and MM are less affected. In an investigation of the creatine kinase activity in 200 human sera, *Gruber* (4) found a mean activating effect of 11%, using a final concentration of 2 mmol/l EDTA; this was interpreted as the influence of Ca^{2+} ions introduced to the test mixture by serum and reagents. Therefore, we also measured the influence of the type of cation chelator (EGTA, EDTA) and the cation chelator concentration on the activities of the different creatine kinase isoenzymes (fig. 2,3). The activation effect of chelators is higher on isoenzyme BB than on isoenzyme MB, and lowest on isoenzyme MM. EGTA shows a greater activating effect than EDTA, and for both compounds, the effect is concentration-dependent.

[1]) Authors and Editorial Office agreed to reserve the publication for this special issue.

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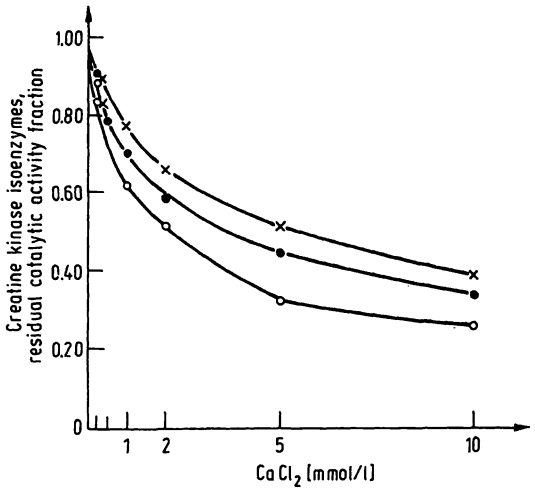

Fig. 1. Influence of Ca^{2+} concentration on catalytic activity of the isoenzymes of creatine kinase.
Catalytic activity in reaction mixture with Ca^{2+} ions is expressed as fraction of that obtained in reaction mixture without addition of Ca^{2+}. Conditions are given in the text. The data given are the means of the duplicates. The standard deviation was estimated by duplicates using the equation $s^2 = \frac{\Sigma R^2}{2m}$ (R = difference between duplicates, m = number of duplicate assays). s was ± 2.2 U/l.
Creatine kinase MM (111.7 U/l) x——x; creatine kinase MB (101.7 U/l) •——•; creatine kinase BB (102.6 U/l) o——o.

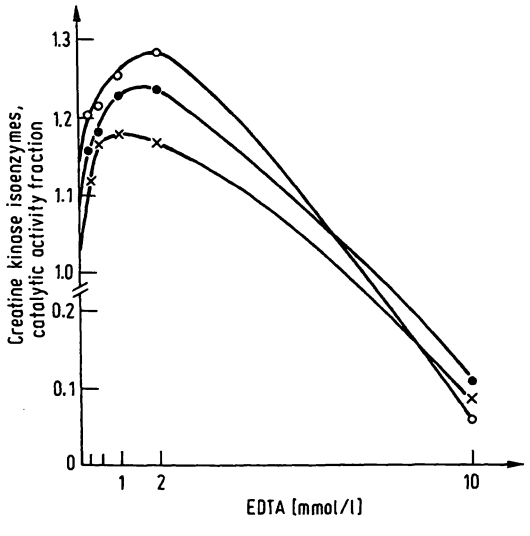

Fig. 2. Influence of EDTA concentration on catalytic activity of the isoenzymes of creatine kinase.
Stimulation or inhibition of the catalytic activity in reaction mixture with EDTA is expressed as fraction of that obtained in reaction mixture without EDTA.
Symbols: see fig. 1. S from duplicate assays was ± 3.3 U/l.

At higher concentrations, the effects of EGTA and EDTA on creatine kinase show further differences. This is because EGTA, in contrast to EDTA, binds especially Ca^{2+} ions and does not interfere with the Mg^{2+} ions necessary for the creatine kinase reaction. At higher

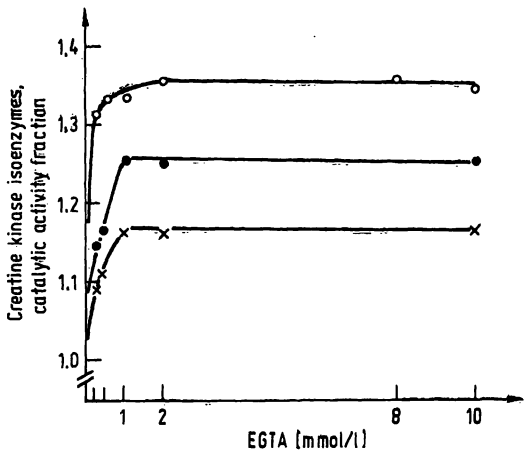

Fig. 3. Influence of EGTA concentration on catalytic activity of the isoenzymes of creatine kinase.
Stimulation of the catalytic activity in reaction mixture with EGTA is expressed as fraction of that obtained in reaction mixture without EGTA. Symbols: see fig. 1. S from duplicate assays was ± 1.4 U/l.

concentrations of EDTA, the concentration of Mg^{2+} ions in the reaction mixture is considerably decreased, creatine kinase activities being reduced to a minimum. In addition, there are differences between the two chelators at lower concentrations; the activating effect of EGTA is higher than that of EDTA, especially on the isoenzyme BB. It seems that the elimination of the inhibitory effect by Ca^{2+} ions is not the only reason for this activating effect.

Discussion

Hitherto, the influence of Ca^{2+} inhibition on the behaviour of the three isoenzymes MM, MB and BB had not been studied. It can be seen that the different influence of Ca^{2+} ions is important, because Ca^{2+} ions may not only result in a falsely low value, they may also lead to the recording of a false relationship between isoenzyme activities. This can lead, for example, to errors in the diagnosis of myocardial infarction when the measurement of percentage MB-isoenzyme activity in relation to the total creatine kinase activity is used as diagnostic aid (9). Knowledge of these different influences is also necessary in the determination of creatine kinase isoenzymes after mini-column chromatography. This technique has gained in importance not only in the fields of muscle diseases and myocardial infarction but also in diseases marked by the appearance of isoenzyme BB in human serum (10, 11, 12). Therefore, the addition of chelators to the reaction mixture may improve the accuracy of activity measurements of creatine kinase isoenzymes and may be useful for facilitating a comparison of results obtained in various laboratories.

In our view, the addition of EGTA at a concentration of 2 mmol/l seems to be more advantageous than adding EDTA (4).

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Dr. K. Jung
Department of Experimental
Organ Transplantation at the
Chair of Urology,
Humboldt University Berlin
Leninallee 49
DDR-1017 Berlin

Dr. W. Liese
Department of Clinical Chemistry
Medical Academy Magdeburg
Leipziger Str. 44
DDR-301 Magdeburg